Synthesis and Biological Activity of Some 4-(Substituted)aminopyrimidines[1]

HAYIM SEGAL,[2] CHARLES HEDGCOTH, AND CHARLES G. SKINNER

The Clayton Foundation Biochemical Institute and the Department of Chemistry, The University of Texas, Austin, Texas

Received February 5, 1962

The isolation and subsequent synthesis of kinetin, 6-(2-furfuryl)-aminopurine,[3] led to the preparation of a large number of 6-(substituted) purines[4,5] which were examined for their physiological activities in a multitude of biological systems. These types of derivatives were found, long ago, to augment the rate of seed germination,[6] and while the structure of the 6-substituent grouping on the purine nucleus could be varied within wide limits,[7] the purine moiety itself may be essential,[8] since a number of comparably substituted pyrazolo-[3,4-d]pyrimidines were inactive. Since this latter heterocyclic nucleus is not of natural origin, it was decided to prepare and determine the biological activity of a number of 4-(substituted)aminopyrimidines, containing the appropriate substituent groupings, which occur in the various biologically active 6-substituted purine analogs.

These 4-(substituted) aminopyrimidines were synthesized through the condensation of the appropriate amine with 2,4-dimercaptopyrimidine[9] to yield the corresponding 4-(substituted)amino-2-pyrimidinethiol. The derivatives then were treated with Raney nickel to remove the mercapto grouping and yield the corresponding 4-(substituted)aminopyrimidine derivatives as indicated in the accompanying equations.

The latter reaction gave the anticipated product in every example except for the hydrogenolysis of 4-furfurylamino-2-pyrimidinethiol, in which the furfuryl group itself was reduced during the reaction to

(1) This investigation was assisted in part by a United States Public Health Service research grant (CY-5779), National Institutes of Health.

(2) Rosalie B. Hite Postdoctoral Fellow 1960–1961; on leave from The Weizmann Institute of Science, Rehovoth, Israel.

(3) C. O. Miller, F. Skoog, F. S. Okumura, M. H. VonSaltza, and F. M. Strong, *J. Am. Chem. Soc.*, **77**, 2662 (1955).

(4) C. G. Skinner and W. Shive, *ibid.*, **77**, 6692 (1955).

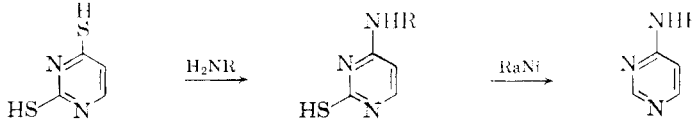
(5) See F. M. Strong, "Topics in Microbial Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 98–159, for a review of these derivatives.

(6) C. O. Miller, *Plant Physiol.*, **31**, 318 (1956).

(7) C. G. Skinner, J. R. Claybrook, F. Talbert, and W. Shive, *ibid.*, **32**, 117 (1957).

(8) C. G. Skinner and W. Shive, *ibid.*, **32**, 500 (1957).

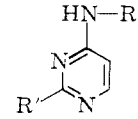
(9) P. B. Russell, G. B. Elion, E. A. Falco, and G. H. Hitchings, *J. Am. Chem. Soc.*, **71**, 2279 (1949).



yield 4-(tetrahydrofurfuryl)aminopyrimidine. Several attempts to isolate the 4-furfurylaminopyrimidine from this reaction sequence failed to produce the desired derivative, and, in view of the fact that the corresponding benzyl analog did not possess significant activity in the seed germination assay as subsequently indicated, no further attempts were made to prepare this derivative. In an effort to increase the water solubility of some of these compounds, the 4-*n*-pentylamino-2-pyrimidinethiol was converted to the corresponding 2-carboxymethylthio analog *via* condensation with chloroacetic acid. In addition, to determine the effect of other structural modifications on the biological activity of the molecule, the latter compound was hydrolyzed under acid conditions to yield the 2-hydroxy derivative, and the biological activities of these compounds were subsequently examined. The ultraviolet absorption spectra of these derivatives are summarized in Table I.

TABLE I

ULTRAVIOLET SPECTRA OF SOME SUBSTITUTED-PYRIMIDINES[a]



		Absorption spectra, mμ[b]						
		pH 1				pH 11		
R	R'	λ_{max}		λ_{min}		λ_{max}		λ_{min}
n-C_4H_9	—SH	233	275	252		260		. .
n-C_5H_{11}	—SH	233	274	252		260		. .
iso-C_5H_{11}	—SH	236	276	254		260		. .
n-C_6H_{13}	—SH	234	273	252		262		. .
n-C_7H_{15}	—SH	235	276	253		260		. .
$C_6H_5CH_2$	—SH	234	275	216	253	260	208	218
$C_4H_3OCH_2$[d]	—SH	232	275	217	251	262	219	228
n-C_5H_{11}	—OH	216	278	241		204	269	250, 228–237[c]
$C_6H_5CH_2$	—OH	207	280	242		201	267	249, 225–234[c]
n-C_6H_{13}	—H	256		207		246	278	265
n-C_7H_{15}	—H	256		210		246		265–282[c]
$C_6H_5CH_2$	—H	256		221		244		221, 266–280[c]
$C_4H_7OCH_2$[e]	—H	255		214		242		262–279[c]

[a] Spectra determined at 25 μg./ml. concentration in water. [b] Spectral range examined: 320–200 mμ. [c] Shoulder. [d] Furfuryl group. [e] Tetrahydrofurfuryl group.

A preliminary study of the microbial toxicity of these analogs in several bacterial systems showed that the more inhibitory derivatives were uniformly those compounds containing a 2-mercapto grouping. For example, the 4-(alkyl)amino-2-mercaptopyrimidines were toxic to growth of *Leuconostoc dextranicum*[10] at a concentration of about 10 μg./ml., and the inhibitions were not effectively reversed by several natural extracts. A subsequent study of these derivatives as antitumor agents using an RC mammary adenocarcinoma implanted in dba mice[11] was carried out, and some inhibitions of tumor growth were observed; *e.g.*, 2-mercapto-4-pentylaminopyrimidine at a dosage of 100 γ/day reduced the rate of tumor growth about 80% under the testing conditions.[11] In general, the other analogs gave values of only about 50% under comparable assay conditions, and their poor solubilities precluded testing them at higher concentration levels.

Using previously reported techniques,[12] the effect of pretreatment of Early Curled Simpson lettuce seed with these 4-(substituted)pyrimidines both alone and in the presence of gibberellic acid on their rate of germination was determined. The lettuce seeds were pretreated with 10 and 30 μg./ml. solutions of the various 4-(substituted) aminopyrimidines for 3 hr. in the dark at 25°; they were then placed in petri dishes on filter paper wet with water and were allowed to stand in the dark at 30° for 36 hr. Concurrently, seeds were also treated with mixtures of these pyrimidine compounds at the indicated concentrations and gibberellic acid at a concentration of 30 μg./ml. At periodic intervals the treated seeds were examined for any visual evidence of enhanced rate of germination over that of seed pretreated in an identical fashion with water alone. None of the analogs produced any observable effect on the rate of germination under either set of experimental conditions. Furthermore, they were relatively non-toxic to the seed since a control experiment conducted at the same time in which the seeds were pretreated and then allowed to germinate in the light produced essentially quantitative germination within 24 hr. In view of the lack of seed germination activity in the series, as well as the lack of activity previously observed in a series of comparable 4-(substituted)pyrazolo[3,4-d]pyrimidines,[8] the purine nucleus would appear to be essential for producing analogs which possess "kinetin-like" activity.

(10) J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954).

(11) C. G. Skinner, G. F. McKenna, T. J. McCord, and W. Shive, *Texas Repts. Biol. Med.*, **16**, 493 (1958).

(12) C. G. Skinner, F. D. Talbert, and W. Shive, *Plant Physiol.*, **33**, 190 (1958),

Experimental[13]

4-*n*-Butylamino-2-pyrimidinethiol.—A mixture of 2.88 g. of 2,4-dimercaptopyrimidine[14] and 4.38 g. of *n*-butylamine was heated under reflux for about 5 hr., cooled, and 50 ml. of water added to the reaction mixture. There was recovered 3.3 g. of product, which was recrystallized from methanol, m.p. 212–214° dec.

Anal. Calcd. for $C_8H_{13}N_3S$: C, 52.42; H, 7.15; N, 22.92; S, 17.50. Found: C, 52.16; H, 6.95; N, 22.71; S, 17.1.

4-Isopentylamino-2-pyrimidinethiol.—A sample of 0.72 g. of 2,4-dimercaptopyrimidine and 2.62 g. of isopentylamine was heated to boiling for 4 hr. After cooling, ether was added, and there was recovered after recrystallization from methanol 0.8 g. of product, m.p. 198–199° dec.

Anal. Calcd. for $C_9H_{15}N_3S$: C, 54.78; H, 7.66; N, 21.30; S, 16.25. Found: C, 54.43; H, 7.58; N, 20.87; S, 15.90.

4-*n*-Hexylamino-2-pyrimidinethiol.—Heating under reflux of 5.76 g. of 2,4-dimercaptopyrimidine and 24 g. of *n*-hexylamine for about 8 hr., followed by cooling, yielded 6.6 g. of product, m.p. 200–204° dec., recrystallized from methanol.

Anal. Calcd. for $C_{10}H_{17}N_3S$: C, 56.83; H, 8.11; N, 19.89; S, 15.17. Found: C, 56.61; H, 7.90; N, 19.53; S, 14.65.

4-*n*-Heptylamino-2-pyrimidinethiol.—A mixture of 5.76 g. of 2,4-dimercaptopyrimidine and 27.6 g. of *n*-heptylamine was heated under reflux for about 8 hr. After cooling, about 500 ml. of ether was added to the reaction mixture, and 6.5 g. of product was recovered, and recrystallized from ethanol; m.p. 179–180° dec.

Anal. Calcd. for $C_{11}H_{19}N_3S$: C, 58.62; H, 8.50; N, 18.65; S, 14.23. Found: C, 58.64; H, 8.92; N, 18.22; S, 14.11.

2-Carboxymethylthio-4-*n*-pentylaminopyrimidine Hydrochloride.—A sample of 5.9 g. of 4-*n*-pentylamino-2-pyrimidinethiol[9] was heated in the presence of 2.83 g. of chloroacetic acid in 35 ml. of water for about 1 hr. After cooling, the solution was reduced to dryness *in vacuo;* the residue was crystallized from methanol-acetone-dioxane to yield 4 g. of product, m.p. 143–145° dec.

Anal. Calcd. for $C_{11}H_{17}N_3SO_2 \cdot HCl$: N, 14.40; S, 10.98. Found: N, 14.40; S, 10.44.

4-*n*-Pentylamino-2-pyrimidinol.—A sample of 1.28 g. of 2-carboxymethylthio-4-*n*-pentylaminopyrimidine hydrochloride was heated under reflux in the presence of 20 ml. of concentrated hydrochloric acid for about 4 hr. After cooling, sufficient ammonium hydroxide was added to adjust the pH to about 7, and there was recovered 0.7 g. of product which was recrystallized from water, m.p. 105–107° dec.

Anal. Calcd. for $C_9H_{15}N_3O$: C, 59.65; H, 8.34; N, 23.19. Found: C, 60.09; H, 8.37; N, 23.00.

4-*n*-Hexylaminopyrimidine.—A mixture of 3.17 g. of 4-*n*-hexylamino-2-pyrimidinethiol, 1.59 g. of sodium carbonate, and about 12 g. of Raney nickel was suspended in 100 ml. of ethanol and heated under gentle reflux for about 24 hr. The hot reaction mixture was filtered, an equal amount of fresh Raney nickel was added, and the system was heated an additional 8 hr. This step was then

(13) All melting points are uncorrected. The authors are indebted to J. D. Glass and Richard Johle for the elemental analyses, and to Mrs. J. Humphreys for assistance with the microbial assays. The ultraviolet spectra were determined on a Beckman Model DB Recording Spectrophotometer.

(14) D. J. Brown, *J. Soc. Chem. Ind.*, **69**, 353 (1950).

repeated, the filtrate was reduced to dryness *in vacuo*, and the residue was crystallized from hexane-benzene to yield 0.8 g. of product, m.p. 61–63°.

Anal. Calcd. for $C_{10}H_{17}N_3$: C, 67.00; H, 9.55; N, 23.43. Found: C, 67.13; H, 9.79; N, 23.39.

4-*n*-Heptylaminopyrimidine Hydrochloride.—The same preparative procedure as indicated above for the *n*-hexyl analog was repeated using 4.5 g. of 4-*n*-heptylamino-2-pyrimidinethiol, 2.31 g. of sodium carbonate, and about 18 g. of Raney nickel suspended in 100 ml. of ethanol. The filtrate from the second hydrogenolysis step was reduced to dryness *in vacuo*, and taken up in dimethylformamide and treated with Darco G-60. After filtering, the resulting solution was treated with hydrogen chloride and ether was added to induce precipitation of the hydrochloride salt. There was recovered 1.35 g. of product, m.p. 147–152° dec.

Anal. Calcd. for $C_{11}H_{19}N_3 \cdot HCl$: C, 57.50; H, 8.77; N, 18.29. Found: C, 57.20; H, 8.61; N, 18.08.

4-Benzylaminopyrimidine.—This analog was prepared by three different synthetic routes. The interaction of 1.44 g. of 2,4-dimercaptopyrimidine and 3.22 g. of benzylamine produced a 98% yield of 4-benzylamino-2-pyrimidinethiol, m.p. 248–250° (reported[9] m.p. 248–249°). A sample of 2.6 g. of this material was treated with Raney nickel as previously described for the analogous 4-(substituted)-amino-2-pyrimidinethiols with additional boiling of the green residue in water and removal of the green oil by gravimetric filtration, and addition of ethanol to the flask to yield 0.70 g. of product which was recrystallized from ethanol–water, m.p. 105–107°.

Anal. Calcd. for $C_{11}H_{11}N_3$: C, 71.33; H, 5.99; N, 22.69. Found: C, 71.09; H, 6.26; N, 22.55.

Treatment of 1.9 g. of 4-benzylamino-2-pyrimidinethiol with 0.84 g. of chloroacetic acid yielded 1.2 g. of 4-benzylamino-2-carboxymethylthiopyrimidine, m.p. 105°; reported m.p. 109–111°.[15] A 1.38 g. sample of the latter derivative and about 7 g. of Raney nickel were suspended in 25 ml. of ethanol and heated under reflux for about 9 hr. The reaction mixture was filtered while still hot, and the filtrate was reduced to dryness *in vacuo*. After recrystallization from ethanol–water there was obtained 0.74 g. of product identical with the dethiolated sample isolated above as evidenced by the lack of depression of a mixture melting point and similarity in three different chromatographic systems.

4-Benzylaminopyrimidine was also prepared by the treatment of 0.5 g. of 4-benzylamino-6-chloropyrimidine[16] with hydrogen at atmospheric pressure in the presence of 0.5 g. of magnesium oxide and 0.5 g. of palladium–charcoal catalyst in dilute ethanol. After about 3 hr., the mixture was filtered, the solvent was removed, and the residue was recrystallized from ethanol–water to yield 0.17 g. of product which was identical with the material previously indicated as evidenced by the lack of depression of a mixture melting point and comparable R_f values in two solvent systems.

4-(2-Tetrahydrofurfurylamino)-pyrimidine Hydrochloride.—A mixture of 1.03 g. of 4-(2-furfurylamino)-2-pyrimidinethiol,[17] 5 g. of Raney nickel,[14] and 0.26 g. of sodium carbonate in 100 ml. of ethanol was treated as described for 4-*n*-hexylaminopyrimidine. After reduction to dryness *in vacuo*, the residual oil was dissolved in 7 ml. of chloroform, filtered, and again reduced to dryness. The result-

(15) G. H. Hitchings and P. B. Russell, *J. Chem. Soc.*, 2455 (1949).
(16) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **80**, 2185 (1958).
(17) D. S. Acker, *Chem. Abstr.*, **52**, 20212i (1958); U. S. Patent 2,844,578 (July 22, 1958).

ing residue was taken up in about 30 ml. of ether and treated with anhydrous hydrogen chloride to yield 0.2 g. of material which was recrystallized from ethanol–ether to yield 0.1 g. of product, m.p. 155–157°.

Anal. Calcd. for $C_9H_{13}N_3O \cdot HCl$: C, 50.11; H, 6.54; N, 19.48. Found: C, 50.30, 50.56; H, 6.52, 6.79; N, 19.88.

Acknowledgment.—The authors are deeply grateful to Professor William Shive for his interest and invaluable advice throughout the course of this study.

COMMUNICATION TO THE EDITOR

C(19)-Substituted Steroid Hormone Analogs. I. 17β-Hydroxy-3-oxoandrost-4-ene-19-nitrile

TIMOTHY JEN AND MANFRED E. WOLFF

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco 22, California

Received March 5, 1962

The intense sodium retaining activity of aldosterone as well as the favorable myotrophic/androgenic ratios of the 19-nortestosterone derivatives shows that modification of the angular methyl groups of steroids can have profound consequences relative to physiological activity. Syntheses of aldosterone[1-4] and 18-nitriloprogesterone[5] from intact steroids by use of intramolecular reactions have been described recently and the preparation of 3β-hydroxycholest-5-ene-19-nitrile[6] and various 6,19[7] and 11,19[8] ethers of saturated steroids has been disclosed.

We wish to report the preparation of 17-hydroxy-3-oxoandrost-4-ene-19-nitrile (XIII), a testosterone analog, using 5α-chloro-6β-hydroxysteroid intermediates. These intermediates are of general utility for the production of C(19) substituted steroids from the

(1) K. Heusler, J. Kalvoda, C. Meystre, P. Wieland, G. Anner, A. Wettstein, G. Cainelli, D. Arigoni, and O. Jeger, *Experientia*, **16**, 21 (1960); *Helv. Chim. Acta*, **44**, 502 (1961).

(2) L. Velluz, G. Muller, R. Bardoneschi, and A. Poittevin, *Compt. rend.*, **250**, 725 (1960).

(3) D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, **82**, 2641 (1960); **83**, 4083 (1961).

(4) M. E. Wolff, J. F. Kerwin, F. F. Owings, B. B. Lewis, B. Blank, A. Magnani, and V. Georgian, *J. Am. Chem. Soc.*, **82**, 4117 (1960).

(5) A. L. Nussbaum, F. E. Carlon, E. P. Oliveto, E. Townley, P. Kabasakalian, and D. H. R. Barton, *J. Am. Chem. Soc.*, **82**, 2973 (1960).

(6) D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet, *J. Am. Chem. Soc.*, **83**, 4076 (1961).

(7) A. Bowers, L. C. Ibañez, M. E. Cabezas, and H. J. Ringold, *Chem. and Ind.*, 1299 (1960).

(8) Ch. Meystre, K. Heusler, J. Kalvoda, P. Wieland, G. Anner, and A. Wettstein, *Experientia*, **17**, 475 (1961).